

# PATENT SPECIFICATION

766,992

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Date of filing Complete Specification : Feb. 18, 1955.

Application Date : March 19, 1954. No. 8172/54.

Complete Specification Published : Jan. 30, 1957.

Index at Acceptance :—Classes 2(3), C2D48: and 81(1), B4.

International Classification :—A61k. C07g.

## COMPLETE SPECIFICATION.

### Improvements in or relating to the Production of Heparin.

We, BOOTS PURE DRUG COMPANY LIMITED, a British Company, of Station Street, Nottingham, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to improvements in the production of alkali metal heparin salt and has for its object the provision of a process for the removal of coloured impurities from heparin salts.

Heparin which may be isolated from animal lung tissues by processes of extraction and purification which are well known in the art, is believed to be a mixture of polysulphuric esters of mucoitin and is a very valuable anticoagulant which is extensively used in clinical machines. Free heparin is acidic in nature and is generally isolated in the form of its salt with an alkali metal, for example, in the form of its sodium salt. Heparin is generally supplied to the medical profession as a solution of the sodium salt which is yellowish or brown in colour. It has long been felt desirable to eliminate this colourisation and various methods have been investigated with a view to treating the alkali metal salts of heparin to obtain a product which would give a colourless solution.

According to the process of our invention the alkali metal salt of heparin is heated in aqueous medium with an alkali metal salt of permanganic acid at a temperature within the range of 60 to 90°C. and at a pH within the range of 8.0 to 8.5 and the solution of heparin salt is then separated from precipitated manganese compounds. A convenient temperature for operation is approximately

80°C., and a convenient strength of heparin salt solution is one containing approximately 5000 units of heparin per cc. The salt of permanganic acid which is used will be determined partly by the nature of the cation which is required to be present in the salt of heparin finally obtained. Heparin is normally prepared in the form of its sodium salt and accordingly for the preparation of this salt sodium permanganate would be employed in the above process. It will be understood however that the invention is not limited to the use of the sodium salt of permanganic acid.

The quantity of permanganate which is required to effect the decolourisation is subject to variation according to the particular batch of heparin salt which is being decolourised. The precise quantity to be employed may be determined by treating aliquots of each batch of heparin salt with increasing quantities of permanganate solution until an amount of permanganate solution is determined which is just sufficient to effect decolourisation under the conditions hereinbefore described. We have found that in general where sodium permanganate is employed it is preferable to use approximately 1 gram-mole of permanganate in order to decolourise 100 million units of heparin salt.

The length of time for which the reaction is allowed to proceed varies according to the nature of the particular batch of heparin salt which is being treated but it is found that under the conditions herein outlined decolourisation of the heparin salt solution is usually complete within 15 to 30 minutes of the addition of the permanganate solution. It is found that little or no loss of

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potency of the heparin occurs during this period of time.

The range of pH within which the reaction is conducted has been found to be critical. Thus if the pH is significantly below 8.0 it is found that decolourisation of the heparin salt can be effected only with difficulty or not at all whilst if the pH is substantially higher than 8.5 the decolourisation is accompanied by a marked loss of heparin due to degradation.

The temperature at which the reaction is performed is also of importance. We have found that if the reaction takes place at room temperature the resulting manganese dioxide is deposited in the form of very fine particles which are extremely difficult to separate by filtration. In fact the manganese dioxide may be deposited in colloidal form and may be so difficult to remove that contamination of the isolated heparin salt occurs.

If however, the reaction is performed at an elevated temperature within the range specified above the manganese dioxide is deposited in granular form and is easily removed from the product. We have found that the reaction is most conveniently conducted at a temperature of approximately 80°C.

When the decolourisation of the heparin salt solution has been effected in the manner described above, the liberated manganese dioxide is isolated by filtration and solid heparin salt is recovered from the filtrate by methods which are well known in the art. Thus, for example, the aqueous filtrate may be poured into an excess of alcohol when the heparin salt is precipitated as a white solid and may be collected and dried.

The decolourisation of heparin in the manner described above also serves partially to remove pyrogens. In order to ensure complete removal of pyrogen from the heparin salt it is necessary to subject the material to further treatment, which may be effected by known methods, e.g. treatment with active charcoal or filtration through a cellulose packing. Alternatively the pyrogens may be removed by the process of our co-pending Application No. 4987/55 (Serial No. 766,993).

The following non-limitative example illustrates the invention.

#### EXAMPLE.

300 g. of heparin (in the form of its sodium salt) of a potency of 100 units per mg. is dissolved in 4 litres of distilled water and the solution is filtered through a bed of kieselguhr, the kieselguhr being washed

several times with small portions (100 c.c.) of distilled water. The volume of the combined filtrate and washings is adjusted to 6 litres by the addition of distilled water and the solution is rapidly heated to 80°C. The pH of the hot solution is adjusted to 8.0—8.5 by the addition of 5N sodium hydroxide solution and 100 c.c. of a 40% w/v solution of sodium permanganate is added with vigorous stirring. The solution is allowed to stand for 30 minutes and the temperature is maintained at 80°C. The product is cooled to approximately 40°C., and the manganese dioxide which has separated is isolated by filtration through a bed of kieselguhr. The kieselguhr filter bed is suspended in 1 litre of distilled water and is heated to 80°C. the pH being adjusted to 8.0—8.5 if necessary. The suspension is cooled to 40°C. and is filtered, the filter bed being washed with 2 portions each of 250 c.c. of distilled water. The filtrate is combined with that from the main bulk of the reaction and the solution is adjusted to pH 6.5. To the solution is added sufficient sodium chloride to produce a concentration of 1% in the final solution. The solution so obtained is clarified by filtration and the filtrate is poured with stirring into four times its volume of 95% alcohol. The solid which separates is isolated by filtration and is dried *in vacuo* at 35°C. There is thus obtained heparin (as the sodium salt) in the form of a white powder which has an activity of approximately 100 units per mg.

What we claim is:—

1. A process for the removal of coloured impurities from an alkali metal salt of heparin comprising heating the heparin salt at a temperature within the range 60 to 90°C. and at a pH within the range 8.0 to 8.5 in aqueous medium within an alkali metal salt of permanganic acid and separating the solution of heparin salt from precipitated manganese compounds.

2. A process as claimed in Claim 1, in which the salt of permanganic acid is sodium permanganate and approximately 1 gram mole of sodium permanganate is used per 100,000,000 units of heparin salt.

3. A process as claimed in Claim 1, and substantially as described in the Example.

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## PROVISIONAL SPECIFICATION.

## Improvements in or relating to the Production of Heparin.

We, BOOTS PURE DRUG COMPANY LIMITED, a British Company, of Station Street, Nottingham, do hereby declare this invention to be described in the following statement:—

This invention relates to improvements in the production of heparin and has for its object the provision of a process for the removal of coloured impurities and pyrogens from heparin.

Heparin is believed to be a mixture of polysulphuric esters of mucoitin and is a very valuable anticoagulant which is extensively used in clinical medicine. It is generally supplied to the medical profession as a solution which is yellowish or brown in colour. It has long been felt desirable to eliminate this colourisation and various methods have been investigated with a view to treating the heparin to obtain a product which would give a colourless solution.

We have now found that the coloured impurities in heparin may be removed rapidly and efficiently without substantial loss of potency of the heparin by the process which is to be described hereinafter. Heparin may be isolated from animal lung tissue by processes of extraction and purification which are well known in the art. The end product of these processes is an off-white or buff coloured powder. According to the process of our invention the coloured powder (which is normally of a potency of approximately 100  $\mu$ /mg. is dissolved in water to give a solution containing approximately 5000  $\mu$ /c.c and the solution so obtained is treated at an elevated temperature, preferably at approximately 80°C. and at a pH which is preferably within the range of 8.0 to 8.5, with a solution of a salt of permanganic acid. The salt of permanganic acid which is used will be determined partly by the nature of the cation which is required to be present in the salt of heparin finally obtained. Heparin is normally prepared in the form of its sodium salt and accordingly for the preparation of this salt sodium permanganate would be employed in the above process. It will be understood however that the invention is not limited to the use of the sodium salt of permanganic acid.

The quantity of permanganate which is required to effect the discolourisation is subject to variation according to the particular batch of heparin which is being decolourised. The precise quantity to be employed may be determined by treating aliquots of each batch of heparin with

increasing quantities of permanganate solution until an amount of permanganate solution is determined which is just sufficient to effect decolourisation under the conditions hereinbefore described. We have found that in general where sodium permanganate is employed it is necessary to use approximately 1 gram-mole of permanganate in order to decolourise 100 million units of heparin.

The length of time for which the reaction is allowed to proceed varies according to the nature of the particular batch of heparin which is being treated but it is found that under the conditions herein outlined decolourisation of the heparin solution is complete within 15 to 30 minutes of the addition of the permanganate solution. It is found that little or no loss of potency of the heparin occurs during this period of time.

The range of pH within the reaction is conducted has been found to be critical. Thus if the pH is significantly below 8.0 it is found that decolourisation of the heparin can be effected only with difficulty or not at all whilst if the pH is substantially higher than 8.5 the decolourisation is accompanied by a marked loss of heparin due to degradation. We have found that the reaction is preferably conducted under conditions such that the initial pH of the reaction mixture is within the range 8.0—8.5.

The temperature at which the reaction is performed is also of importance. We have found that if the reaction takes place at room temperature the resulting manganese dioxide is deposited in the form of very fine particles which are extremely difficult to separate by filtration. In fact the manganese dioxide may be deposited in colloidal form and may be so difficult to remove that contamination of the isolated heparin occurs.

If however, the reaction is performed at an elevated temperature the manganese dioxide is deposited in granular form and is easily removed from the product. We have found that the reaction is most conveniently conducted at a temperature of approximately 80°C. but it will be understood that our invention is not limited to the use of this particular reaction temperature.

When the decolourisation of the heparin solution has been effected in the manner described above, the liberated manganese dioxide is isolated by filtration and solid heparin is recovered from the filtrate by methods which are well known in the art. Thus, for example, the aqueous filtrate may

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be poured into the excess of alcohol when heparin is precipitated as a white solid and may be collected and dried.

Accordingly our invention consists in a process for the decolourisation of heparin which is characterised by the step of reacting an aqueous solution of heparin at a pH within the range of 8.0 to 8.5 and at an elevated temperature, preferably at approximately 80°C., with an aqueous solution of a salt of permanganic acid, the cation of which salt is preferably identical with the cation of the heparin salt which is finally to be isolated, in such an amount as is hereinbefore defined.

The decolourisation of heparin in the manner described above also serves partially to remove pyrogens, such removal being completed by subsequent treatment of the heparin, e.g. as described in co-pending Application No. 4987/55 (Serial No. 766,993).

The following non-limitative example illustrates the invention.

#### EXAMPLE.

300 g. of heparin of a potency of 100  $\mu$ /mg. is dissolved in 4 litres of distilled water and the solution is filtered through a bed of kieselguhr, the kieselguhr being washed several times with small portions (100 c.c.) of distilled water. The volume of the combined filtrate and washings is adjusted to 6 litres by the addition of distilled water and the solution is rapidly heated to 80°C. The pH of the

hot solution is adjusted to 8.0—8.5 by the addition of 5N sodium hydroxide solution and 100 c.c. of a 40% w/v solution of sodium permanganate is added with vigorous stirring. The solution is allowed to stand for 30 minutes and the temperature is maintained at 80°C. The product is cooled to approximately 40°C. and the manganese dioxide which has separated is isolated by filtration through a bed of kieselguhr. The kieselguhr filter bed is suspended in 1 litre of distilled water and is heated to 80°C. the pH being adjusted to 8.0—8.5 if necessary. The suspension is cooled to 40°C. and is filtered, the filter bed being washed with 2 portions each of 250 c.c. of distilled water. The filtrate is combined with that from the main bulk of the reaction and the solution is adjusted to pH 6.5. To the solution is added sufficient sodium chloride to produce a concentration of 1% in the final solution. The solution so obtained is clarified by filtration and the filtrate is poured with stirring into four times its volume of 95% alcohol. The solid which separates is isolated by filtration and is dried *in vacuo* at 35°C. There is thus obtained heparin in the form of a white powder which has an activity of approximately 100  $\mu$ /mg.

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